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THE LABORATORY DIAGNOSIS OF HELMINTHIC INFECTIONS*

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Introduction

With the sending of our troops into tropical and subtropical zone has come the threat of an increasing incidence of tropical disease. The medical corps of the Army and Navy are well aware of this and have intensively trained their men in the prevention, diagnosis, and treatment of these diseases. Medical schools are placing more emphasis on these subjects, civilian practitioners are reading more about them in the current journals, and we may be called upon to diagnose more of them by laboratory methods in the future than we have in the past. Any man, in any part of the world today, is within only a few hours flying time of the United States and we may expect to see men return from the war zones symptom free, and develop symptoms only after their arrival here. This means that we must be on our guard for those infections which heretofore, we have rarely seen or which we have diagnosed only from the classroom slide or textbook drawing.

The most insidious of these diseases are the helminthic infections. Historically, there is good evidence that they are as old or

*Presented at the Annual Meeting of the American Society of Medical Technologists, June, 1944, Chicago, Ill.

older than man himself and have undergone morphological changes through the years to reach the highest form of parasitic existence. The Ebers Papyrus, written about 1600 B.C., records a disease caused by a "bowl worm" which it names the "Heltu" and which, by further description was probably the Taenia saginata. Moses, who learned his medicine from the Egyptian priests, laid down sanitary laws to protect his people from infected flesh and separated the animals as "clean" and "unclean" on the basis of whether or not they had visible parasites. The tapeworm is mentioned by Aristotle and the Echinococcus by Hippocrates. Avicenna, an Arab physician (981 A.D.-1037 A.D.), described four kinds of worms: (1) Long worms, probably the *Ascaris Lumbricoides;* (2) Flatworms, pumpkin seed shaped and found in infected beef which were probably *Taenia saginata;* (3) Small worms, probably *Enterobius vermicularis,* and (4) Roundworms, probably the hookworm, *Anklyostome duodenale.*

Some of these helminths have been highly endemic in the Southern parts of the United States for years with as high as 50% of the people in an area infected, but the clinical symptoms are often very slight and may go undiagnosed for years. These infections can, however, become severe or fatal and are always detrimental and often complicated by secondary infection.

Prevention and treatment depend on diagnosis and the diagnosis most often falls in the hands of the civilian technician, the pharmacist's mate or the technical sergeant. This is one of the technician's greatest responsibilities as it is one of the few instances in which he makes a definite diagnosis.

In our routine examination of the urine, sputum, blood, transudates, exudates and tissues, we must be constantly on our guard for these parasites and their ova, but we will seldom be asked to look specifically for them in any of these specimens. Most of our diagnosis will be made from a thorough examination of the feces and it is to this examination that we will confine our discussion.

Examination of the Feces

In the feces the ova of the following may be found: *Ascaris lumbricoides, Necator americanus, Anclyostoma duodenale, Trichuris trichuri, Enterobius vermicularis, Strongyloides stercoralis, Schistosoma mansoni, Schistosoma japonicum, Clonorchis sinensis,*

Fasciola hepatica, Fasciolopsis buski, Taenia saginata, Taenia solium, Diphyllobothrium latum, Hymenolepis nana, Hymenolepis diminuta, Dipylidium caninum, Opisthorchis felineus and Paragonimus westermani.

Collection of Specimens

A small representative sample is all that is required and any clean, cheap, watertight container that can be destroyed after the examination is theoretically good. It is recommended by some workers that a small metal salve box with a cardboard label be used, and for mailing purposes this is certainly the container of choice, but there are certain objections to such boxes: (1) Metal is being conserved and they are difficult to obtain at this time; (2) Fecal specimens are nearly always collected and sent to the laboratory by the patient, the nurse or the orderly and they seldom realize that a small sample is all that is needed and will fill these boxes to the brim or to overflowing. When the top is then placed on the container, the contents will either run down the outside and be a source of infection to all who handle it or stick between the lid and the container and make removal of the metal lid a difficult and messy task; (3) Others will go to the opposite extreme and send only the smallest dab, so that any attempt to see the adult worms is futile.

These objections have been overcome by using a half pint, heavy cardboard, ice cream carton. These are available and cheap, are easily destroyed by burning, and because of the large size, there is a tendency for the collector to send an adequate, but not overflowing sample. These containers are also good for the collection of sputum.

For *Enterobius* infestations it is necessary to collect and examine the perianal scrapings as most of the eggs will be found in this location.

Two types of anal swabs have been found to be suitable:

(1) NIH Swab (Mall, 1937)—A glass rod, which is held in a rubber stopper so that it can be carried in a test tube, is tipped with a small piece of cellophane held in place by a rubber band. The perianal region is swabbed and the cellophane removed from the rod, spread on a glass slide in a few drops of sodium hydroxide,

covered with another slide and pressed together. Examine under low power of the microscope.

(2) Scotch Cellulose tape swab (Graham, 1940)—A piece of Scotch cellulose tape of half-inch width and about 8 cm. long is folded down for about a centimeter at each end, adhesive surfaces together, to form two grips for handling. This ribbon of cellulose is held in forceps in a loop with adhesive surfaces on the outer side and is patted down on the perianal skin. The adhesive surface picks up scales, fecal particles and ova, if any be present. Then place the tape lengthwise on a microscope slide, smooth down with the sides of the forceps and examine with a 16 mm. objective.

Jacobs (1942) has used the Scotch tape on the end of a tongue blade with good results.

Gross Examination

Look for adult worms, larvae, or detached segments. These may be washed in saline and placed in a Petri dish which is then placed on a black background and viewed with a hand lens. The black paper in which X-ray films come wrapped makes an excellent background.

For differentiation of segments of *Taenia saginata* and *Taenia solium*, place in a mixture of equal parts glycerin and 95% alcohol and leave exposed to evaporate. In several hours or overnight the segments will have cleared enough to demonstrate the uterus and make a differentiation.

Preservation of adult helminths:—Do not fix flukes or tapeworms in formalin as is so often done. Relax them first by shaking in physiological salt solution or placing in the ice box for one to several hours. When relaxed, pipette off all the water from the dish and suck up the remaining water with a piece of paper towel, but do not let the parasites dry. Add cold Bouin's fixative drop by drop to the top of the specimen, taking care not to wash the specimen free from the flat surface. After about two minutes of fixation on the surface, add the bulk of the fixative, enough to cover the specimen, and let stand for 12 to 24 hours. Then wash in 50% alcohol for 15-30 minutes, transfer to 70% alcohol and make several changes at intervals of an hour or more until all the yellow picric acid color is gone. Store in 80% alcohol until ready to stain. When ready to stain, run back down the alcohols

using first 70%, then 50%, then lower dilutions until water is reached. The best stains to use are: (1) Carmalum (Mayer's Carmalum Stock Solution 7 cc; glacial acetic acid 0.3 cc; distilled water 100 cc) or (2) Haemalum (stock solution 7 cc; glacial acetic acid 0.5 cc; distilled water 100 cc). Stain slowly for 24 to 36 hours. If fixation with Zenker's solution is preferred, wash the worm several hours in water, then run up through 20%, 40%, 60%, 80% alcohols. When in 80% add iodine, enough to give a port wine color. Leave in this about four hours to allow the iodine to remove the $HgCl_2$ crystals. Wash in fresh 80% alcohol until the iodine color is removed and store in 80% alcohol until ready to stain as before. Mount in balsam.

Direct Smear

Place a drop of physiological saline on a clean slide. Pick out a fleck of feces with a toothpick and thoroughly emulsify it in the saline and spread it over an area of about ¾-inch by 1½ inches. On half of this film place a clean coverslip and, in order to look for protozoan cysts at the same time, place a drop of iodine solution (D'Antoni's is recommended, but Lugol's will do) on the other half and also cover. The film should be thin enough for newspaper to be read through it and the coverslip should be lowered slowly to avoid bubbles. The slide should be examined with the low power (16 mm) objective to look for ova and use the high power (4 mm) objective only for close identification and for protozoa.

Concentration Methods

(1) Willis technic (1921)—Place .5 to 1 gm. of feces in a glass cylinder about 2.5 cm. in diameter and thoroughly emulsify it with 10 to 20 volumes of saturated salt solution (Sp. gr. about 1.200) filling the cylinder so that a meniscus forms at the top. Add the salt solution slowly at first, while stirring, to get complete comminution. On this meniscus place a clean fecal slide (75 x 37 mm) and allow it to remain for 30 minutes. Remove the slide, invert and examine microscopically.

(2) Centrifugalization—Thoroughly mix 1 to 10 gms. of feces in 10 to 20 times its volume of water. Strain through a dampened cheesecloth or wire basket into centrifuge tubes with round bot-

toms. Centrifuge for about 2 minutes at 2500 r.p.m. Decant the supernatant fluid and repeat until it is clear, stirring between runs. Pour off the fluid and examine the sediment.

(3) Clayton Lane Technic—D.C.F. Method (1923)—Measure accurately 1 ml. of specimen and place in a special ground top centrifuge tube. Fill with tap water to within 1 inch of top. Thoroughly comminute by inserting 4 or 5 applicator sticks in the tube and rapidly twisting them between the fingers. Centrifuge at 1000 r.p.m and pour off the supernatant fluid. Nearly fill tube with sodium chloride solution (specific gravity 1.200). Again stir with applicator sticks until the suspension is homogeneous. Return tube to centrifuge, fill to the brim with more brine and cover with a thick cover glass which is secured by the four horns of a special holder. Centrifuge 1 minute at 1000 r.p.m., remove cover glass, place it film side down on cone shaped plasticine supports and examine as a hanging drop.

(4) Simple Sedimentation—Thoroughly mix the whole fecal specimen in 10 to 20 times its volume of tap water and let settle for an hour or two. Cone shaped graduates of pint volume are recommended. Pour off the top 2/3 of the liquid and its debris, fill again with water, mix and allow to settle. Repeat until the supernatant liquid is clear. Pour off water, remove some of the sediment and examine. This method may be modified by allowing the mixture to stand for 24 hours to allow for hatching of miracidia and then examining the upper layers of the liquid for these free swimming forms.

(5) Faust Zinc Sulfate Centrifugal Flotation Technic (1938-1939)—Make a suspension by using one part of specimen, about the size of a pecan, with about 10 parts of lukewarm tap water. Strain about 10 ml. of the suspension through one layer of wet cheesecloth in a small funnel into a test tube. Centrifuge 46 to 60 seconds at top speed. Pour off the supernatant fluid, add 2 or 3 ml. of water, break up sediment by shaking or tapping, and add more water to fill the tube. Repeat until supernatant liquid is clear. Pour off last supernatant fluid, add 3 to 4 ml. of zinc sulfate solution of specific gravity of 1.180 (33% solution of $ZnSO_4.7H_2O$), break up the packed sediment, and add more zinc sulfate to within about a half-inch of the rim. Centrifuge for 45 to 60 seconds at

top speed. Several loopfuls of diagnostic material floating on the surface film are removed with a bacteriological loop onto a clean slide, add one drop of D'Antoni's iodine stain and agitate the preparation manually to insure uniform staining. Mount with a coverglass and examine.

Modifications of this method have been introduced by Otto, Hewitt and Strahan in 1941 in which they use the same zinc sulfate (sp. gr. 1.180) as a direct flotation medium without screening the comminuted feces through cheesecloth.

Summers (1942) modified the method by using zinc sulfate of specific gravity of 1.200 (about 37% solution of $ZnSO_4.7H_2O$) and without straining.

Summary of the Advantages and Disadvantages of the Various Technics

With so many well recommended procedures to be found, it is difficult for the technician to decide which ones to use in routine practice and it is obviously impossible to use all of them. The procedures chosen must be adequate for all types of helminths but not too laborious and time-consuming.

This field is still open to research and there is still a great deal of controversy on the subject, but the weight of evidence at the present indicates that the following general statements are true.

(1) No ONE procedure yet devised is sufficient to adequately diagnose ALL helminthic infections and a combination of two or more procedures seems advisable.

(2) Gross examination is important because adult worms may be present and not be passing ova in the feces.

(3) The direct smear is simple, rapid, almost as good as centrifugalization alone, and may be examined for protozoa and cysts at the same time. However, it requires 500 ova per gram of feces for the hookworm to be diagnosed by this method, unless multiple smears are made.

(4) Centrifuging and sedimentation afford very little concentration and their usefulness is limited mainly to the heavy and operculated eggs, and several direct smears are usually just as good.

(5) Flotation methods are superior to other methods as a means of concentration except in the following cases:

a. Flotation methods are good for nematode and hymenolepis eggs, but are not good for those of *Taenia* or the *Flukes*.

b. Brine flotation methods shrink protozoan cysts and *Schistosome* eggs, but are as good or better than zinc sulfate for some of the helminths (hookworm).

c. The large operculated ova of *Diphyllobothrium latum*, *Fasciola*, and *Fasciolopsis* open up or shrivel and sink in salt solution.

d. The smaller, thick shelled ova of *Clonorchis*, *Metagonimus*, and *Dicrocoelium* are of high density and will not rise to the surface.

(6) Zinc sulfate flotation methods (sp. gr. 1.180) are superior to all others in concentrating at one time helminth eggs, larvae, and protozoan cysts in a diagnosable and visable state.

(7) Loop removal of ova from the surface film is equally as good as touch removal in centrifugal flotation technics.

(8) Straining of the feces has no great merit, is time-consuming and may even lower the efficiency of the flotation method.

(9) In all cases, the feces must be thoroughly mixed in the solution for good flotation results.

(10) Anal swabs (either NIH or Scotch tape types) are best for finding Enterobius vermicularis.

Conclusions

With the above summary as a basis, the following combination of procedures is recommended for routine use in examing the feces for ova and parasites.

1. Gross examination.
2. Direct smear.
3. Zinc sulfate centrifugal flotation (sp. gr. 1.180) with loop or touch removal (Faust method or its modifications, with elimination of the straining procedure, is recommended).
4. Anal swabs (NIH or Scotch tape) for Enterobius.

Success with any of these methods depends also upon the technician's training, perseverance and care in performing the method used. For this reason, some technicians will get good results with a method while others will fail.

Suggestions for Teaching and Practice

It is very discouraging to both teacher and student to try to

demonstrate or practice fecal examinations unless you have some positive specimens available, and in certain seasons of the year and in areas where these parasites are infrequent, these will not be found often in routine examinations. This difficulty may be overcome by going to the local dog pound, the zoo, or the slaughter house and taking samples from the stools of several animals. There is always a high incidence of helminthic infestation among these animals and, although the species of the parasite may differ slightly from the ones infecting men, the stages are easily recognizable or indistinquishable from the human forms and suitable for practice. Make permanent mounts of the typical ones you find and use them for future teaching purposes.

Many artefacts and miscellaneous objects are sources of error in examining the stool microscopically. Notorious among these are the intestinal yeasts and fungi, pollens, vegetable and animal cells (leucocytes, epithelial cells, and muscle fibers), starch granules, oil globules and air bubbles. Pictures of these artefacts are to be found in most of the Standard texts. The large eggs of mites (from food infested by mites) may be present in the stool. Errors may also arise from examination of feces passed on the ground which may be contaminated by worms or eggs of soil nematodes. Only by experience and practice can a satisfactory differential be made.

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BRUCELLOSIS ISOLATED FROM THE BONE CAVITY OF LEFT ILIUM

By ROBERT BARTON COWAN, M.T.

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The history of Brucella infection in man, cows, goats and swine and the nature of the disease have been presented on many occasions and need no review at this time. There are, however, certain aspects of the disease that call for further discussion. One of these is the course of the disease when it breaks down the bone marrow.

The review of the literature on this subject would lead one to believe that this is a rare clinical pathological entity. Hardy[1] reports a case wherein the patient after 30 days had a low grade fever and in the right hand developed teno-synovitis and experienced joint discomfort and stiffness. This inflammation of the synovial membrane and sheath of the tendon is marked by the formation of a tense, painful swelling due to the presence of a fluid effusion within the synovial cavity. His serum agglutinated the Brucella organism.

Hardy reports another case[2] of suspected osteomyelitis of the pelvis of a 12-year-old boy. He had a limp on his right side followed by rigors, pain of right hip, headache, and temperature of 104 degrees. After a surgical operation the only significant finding was a small amount of pus. This revealed no pathogenic organism and it wasn't until a month after his discharge from the hospital that he had another fever and at this time the blood agglutination was taken and found to be positive for Brucella in a high dilution of 1-5120.

A review of recorded cases appears to indicate that the liver and spleen are most commonly affected by the Brucella organism, therefore this is one of the rare cases in medicine of this organism breaking down the bone tissue.

The patient cited in this report was ill for five years or more and had profuse sweats and chills with secondary anemia, severe head-

ache, and generalized aching pain. The laboratory test at this time showed the patient's serum to be positive with Brucella antigen at a high agglutination of 1-640.

The patient was treated for six months for undulant fever and the agglutination test was down to 1-40 at the end of this period.

One year later the agglutination test went up to 1-160 and the patient had a fever again and complained of severe pain in the hip and left axilla. The white cell count was 15,700 with 11 stabs, 75 polys, 24 lymphocytes and one monocyte. The red cell count was 3,870,000. The patient went to the hospital and radiographic examination of the pelvis showed a large cavity in the body of the left ilium. Several pints of pus and fluid were drawn off and cultured on chocolate agar and tryptose dextrose agar. After five days, growth occurred on the tryptose agar plate, later sub-cultured on chocolate agar. The organisms were grown in 10% CO_2 at 37° C. They grew faster on tryptose media than on chocolate agar, though subsequent transfers improved their growth on chocolate. They grew the best without 10% CO_2 and they stained negative and were very small coccobacilli. Some showed polar staining and more so when first isolated. After further examination the organism isolated did not ferment any carbohydrates and indole was not produced. The colonies were small dew drop, smooth, glistening and almost colorless. They were non-capsulated and non-motile.

Twenty c.c. of the patient's blood cultured in liver infusin broth did not show growth of any organisms.

In this long continued case of Brucellosis it localized in two particular areas. The first examination showed the infection in the left ilium and later two tests were run on the right ilium and left axilla. The right ilium showed no growth of Brucella but from the left axilla was isolated the same organism as the one from the left ilium.

At this time the skin test of the killed suspension given to the patient showed an area of six inches in diameter in twenty-four hours.

One known antiserum of Brucella of high titre was run against the unknown organisms as the antigen. This was the final step in the identification and verified the results of the carbohydrate

fermentation. The known antiserum of Brucella had a titre of 1-2500 and agglutinated the unknown bacterium 1-2480.

According to Huddleson's[3] differentiation dyes it has been found that by taking advantage of the behavior of Brucella toward thionin and basic fuchsin in suitable dilutions in a medium, all strains fall into one or the other of three groups. On final examination with bacteriostatic dyes the dilution used of basic fuchsin was 1-100,000 and of thionin 1-200,000. The culture plated on tryptose agar plus thionin gave excellent growth but the organism plated on tryptose agar containing basic fuchsin did not grow, thus showing the organism herein isolated to be Brucella suis. (The organism was sent later to the Iowa State Laboratory and they also identified the organism as Brucella suis.)

The finding of the porcine strain of the organism accounts for the rather severe clinical type of the disease, it being quite generally considered that the porcine strain is more pathogenic for man than the bovine strain[4].

The tryptose agar used was Difco's Tryptose Agar.

The formula used for chocolate agar was the following:

No. 1

Glucose	2.5	grams
Peptone	5.0	"
NaCl	1.25	"
Nutrose	0.5	"
Gelatin	5.0	"
Soluable starch	2.5	"
Agar	7.0	"
Heart broth	185	cc.
Brain broth	65	cc.

Adjust to pH 7.8

No. 2

H_2O (Distilled)	165	cc.
Whole Blood (beef)	35	cc.

After autoclaving both, number two was mixed to number one and plates were poured.

Summary

The case described is a rare and unusual one in which the Brucella organism, variety suis, destroys the bone tissue in several localities of the body. The porcine strain of Brucellosis gives rise to a more severe clinical type of disease and is more pathogenic for man than the bovine strain. One or two other exceptional cases are described along with the isolating of this particular porcine strain of Brucella.

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THE EFFECT OF MENSTRUATION ON THE SEDIMENTATION RATE OF THE RED BLOOD CELLS*

By JOSEPH M. SCOTT, M.T. (ASCP)

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The effect of menstruation on the sedimentation rate of the red blood cells was first studied by the author in 1939 on 26 women, most of whom had tuberculosis, using the Cutler method (1). The results of that series showed that there was little or no difference in the averages of the sedimentation rates determined during either the menstrual or the intermenstrual periods. The one-hour sedimentation rate determined during the period varied from 5.5 mm. lower to 5 mm. higher than the corresponding rates determined in the postmenstrual period, but curiously the average of all tests taken during either period was the same, 14.9 mm.

In 1942-43, while a comparative investigation of the Cutler and Westergren sedimentation methods (2) was being carried out the subject was again taken up, but this time both techniques were used simultaneously.

Literature

Although the fact that menstruation may affect the sedimentation rate (henceforth abbreviated SR) is frequently mentioned in articles dealing with the test there appear to be few papers in which the subject has been specifically investigated and actual findings reported. The fact that it does affect the SR was first noted early in the 1920's at a time when the sedimentation test was being widely used in the investigation of many different diseases.

One such paper is that of Greisheimer (3) who investigated the SR in healty male and female university students. Using the Cutler method she found that on the average the SR was slightly lower during the menses than during the intermenstrual periods. She found that the average sedimentation index for those men-

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struating was 6.4 mm. in an hour (21 women), while for those who were not it was 7.6 mm. (70 women). In no case did she find the rate faster during the menses than at some time between the periods. Her conclusion was that the sedimentation rate in normal women was not affected by menstruation.

A more recent investigation is that of Greene (4) who did Westergren sedimentation tests on every day of the menstrual cycles of 10 healthy women and found no significant variations in the SR at any phase of the cycle. Only three women showed a more rapid SR during the menses than during some part of the intermenstrual period. Lockett (5) found an increase in the SR which reached its maximum just before the commencement of menstruation.

Cutler (6) gave his normal sedimentation rate for healthy women as 2-10 mm. per hour but stated that during menstruation it sometimes went as high as 12 mm.

Linzermeier (7) stated that in normal women it usually required five or six hours for the blood cells to settle to the 18 mm. mark but that during menstruation this time was sometimes reduced to as little as three hours. Nees (8) found that in adult women it took from 13 to 16 hours for the cells to reach the 24-mm. mark but that during the menstrual period the time was sometimes reduced to 10 hours.

Plass and Rourke (9), investigating healthy young women, could not find any definite relationship between the SR and the various phases of the menstrual cycle in which the blood was obtained. They found that the SR varied only slightly from week to week and therefore did not think that menstruation had any significant effect on the SR in healthy women.

Walton (10) studied the SR on six women during different phases of the menstrual cycle. Although the red cell count remained constant the SR was found to fluctuate in a rhythmical manner throughout the cycle. In several patients with pulmonary tuberculosis a rise was noted in the SR during the premenstrual phase, while during the period of flow a sudden diminution in the rate was observed, the rate slowly rising again until the next premenstrual phase was reached. He believed that such fluctuations were of little importance in normal women, since the rises observed were

still within the normal range, but thought they should be taken into consideration in patients in whom the progress of pathological lesions were being recorded.

Hunt (11) found that the SR became more rapid as the fibrin content of the blood increased, and observed further that the fibrin content of the blood was higher during menstruation and pregnancy, which according to him, accounted for the higher sedimentation indices found in these conditions.

Wintrobe and Landsberg (12) in studying the sedimentation phenomenon by means of the Wintrobe hemocrit found a wide range of rates in normal women medical students and nurses (0 to 20 mm.) They found the mean SR to be 10.2 mm. during the first postmenstrual week; 9.3 mm. during the second; 8.6 mm. during the third, and 10.9 during the fourth week. They noted the slight increase preceding and following menstruation but did not think it had any special significance.

Nichols (13), after an exhaustive survey of factors affecting the sedimentation test, merely stated that some find an increased SR during menstruation while others doubt that there is any increase. Stemmler (14) believed that the sedimentation time in healthy women was unaffected by this phenomenon.

Muller (15) summarized the findings of several authorities in her recent monograph but did not add any new material on the subject from her exhaustive analysis of blood examinations in tuberculosis.

Whitby and Britton (16) recommend that the sedimentation test be not done during the menses, or for a few days before and after, due to the fact that the SR is slightly increased at this time. Obermer (17) believed that the SR was consistently increased in some women during the menses, but unaffected in others, and for this reason considered it wiser not to perform the test at this time.

Outline of Study

The patients in the series reported here were undergoing treatment for tuberculosis and were selected in the course of doing routine sedimentation tests on them. If, on questioning, a woman

was found to be menstruating not only was the regular Cutler test done but simultaneously a Westergren test.

Both tests were carried out according to the techniques given in detail in a former paper (2) which will not be further described. Readings were recorded at the end of one, two and twenty-four hours.

In almost all cases the length of time since the commencement of the period, or since its termination, in relation to the time of the sedimentation test, was ascertained. The first tests were done on the first to fifth day of the period with the average time about 2.6 days after its commencement.

The tests were repeated simultaneously on each woman at intervals which ranged from 4 to 11 days after the first tests and averaged 6.8 days. They were done 1 to 8 days after menstruation had ceased, or after an average of about 3.6 days.

Sedimentation tests were done on 26 different women. All had a single series of tests, except one who had a second series. All patients had pulmonary tuberculosis; in five it was minimal, in eight moderately advanced, in ten far advanced, and three had pleurisy with effusion. Their ages ranged from 18 to 34 and averaged just under 25 years. Six women were married, 18 unmarried.

Results

Much of the data on which this study is based is presented in table 1, and the findings are summarized in tables 2 and 3. It will be noted that only one patient, Mrs. D. C., had more than one series of tests. Only the one-hour readings are given in table 1 since they are generally considered more significant than those taken at longer intervals, though reference will be made later to the two-hour and 24-hour readings.

TABLE 1

ONE-HOUR SEDIMENTATION INDICES DURING MENSTRUATION AND WITHIN ONE WEEK AFTER AS DETERMINED SIMULTANEOUSLY BY THE CUTLER AND WESTERGREN METHODS

Serial Number	Case	Age	Marital State	Cutler Sedimentation Index		Westergren Sedimentation Index	
				During Mens.	After Mens.	During Mens.	After Mens.
				mm.	mm.	mm.	mm.
1.	A.K.D.	19	S.	5	7	7	7
2.	M.V.F.	22	S.	6	4	6	10
3.	M.L.	29	S.	8	5.5	19	10
4.	M.D.	32	M.	8	8.5	18.5	11.5
5.	A.I.E.	33	S.	9	5	19	7.5
6.	E.M.M.	34	M.	9	7	15.5	17
7.	J.L.T.	18	S.	10	7.5	24	14
8.	I.H.	19	S.	11	13	27.5	26
9.	M.P.	32	S.	11	8.5	23	11
10.	G.C.	22	S.	14.5	13.5	41.5	35.5
11.	F.K.	19	S.	15	9.5	33.5	22.5
12.	A.E.N.	20	S.	15.5	17	34	32
13.	H.M.M.	27	S.	16	14	42	36.5
14.	E.W.	20	S.	17	11	36	30
15.	E.C.	21	S.	17.5	12	37	26
16.	M.B.	19	S.	17.5	13.5	63.5	26.5
17.	A.R.C.	30	M.	17.5	17	45	38
18.	J.A.	28	S.	17.5	18	35	27
19.	M.B.D.	25	S.	22	24.5	53.5	67
20.	M.M.M.	21	S.	22	20	42	29
21.	E.H.	27	M.	22.5	19	72	56
22.	S.L.M.	19	S.	22.75	25	47	64
23.	A.S.M.M.	26	M.	23	22	51	47
24.	M.F.F.	20	S.	25	28	77	72.5
25.	D.C.	31	M.	26.5	25.5	89	77.5
26.	D.C.*	31	M.	28	30.5	97.5	104
27.	M.J.B.	29	S.	30	26.5	98	87

*Repeated test on same patient at a later date.

TABLE 2

THE BLOOD SEDIMENTATION RATE IN RELATION TO MENSTRUATION

Sedimentation Rate in Relation To Menstruation	Patients Number	Percentage
One-Hour Readings		
Sedimentation Rate Increased during period as shown by one or both tests	23	85.2
Sedimentation Rate Decreased during period as shown by one or both tests	4	14.8
Totals	27	100.0

TABLE 3

THE SEDIMENTATION RATE DURING MENSTRUATION COMPARED WITH THE SEDIMENTATION RATE AFTER MENSTRUATION

SR During Menstruation Compared to SR After Menstruation	Cutler Tests During Mens.	Cutler Tests After Mens.	Westergren Tests During Mens.	Westergren Tests After Mens.
Averages				
	mm.	mm.	mm.	mm.
Average of one-hour readings on 27 patients	15.6	14.9	42.7	36.1
Average difference	...	—0.7	...	—6.6
Differences				
	mm.		mm.	
Range of difference between two comparative tests	—3 to +6		—17 to +377	
Least difference between two comparative tests	0.5		0	
Greatest difference between two comparative tests	6		37	

Table 2 reveals that the sedimentation rate was increased during menstruation in 23 patients, or 85.2 per cent, as shown by one or both methods, while in 4, or 14.8 per cent, it was decreased. It should be pointed out, however, that there was not complete agreement between the results of all the Cutler and Westergren tests; in seven cases one method showed a decrease while the other showed an increase. In one case the Westergren method showed no change between two corresponding tests.

The averages for the one-hour readings are given in table 3. In the Cutler tests the average SR during menstruation was 15.6 mm., while after the period it was 14.9 mm., an average decrease of 0.7 mm. In the Westergren tests the average SR during menstruation was 42.7 mm., while after the period it was 36.1 mm., an average decrease of 6.6 mm.

Table 3 also shows that comparative tests during and after the period show differences ranging from 0.5 to 6 mm. for the Cutler method and 0 to 37 mm. for the Westergren. With the Cutler method the first tests (menstrual) ranged from 3 mm. less to 6 mm. more than the second tests (postmenstrual); while for the Westergren the range was from 17 mm. less to 37 mm. more.

TABLE 4

A COMPARISON OF THE CUTLER AND WESTERGREN METHODS IN REVEALING CHANGES IN THE SEDIMENTATION RATE DURING MENSTRUATION

Changes in Sedimentation Rate During Menstruation	Number of Tests 1-Hr.	2-Hr.	24-Hr.
Cutler Method			
Sedimentation rate INCREASED	18	13	11
Sedimentation rate DECREASED	9	10	9
No change shown in sedimentation rate	0	2	4
Data missing	0	2	3
Totals	27	27	27
Westergren Method			
Sedimentation rate INCREASED	21	20	16
Sedimentation rate DECREASED	5	5	8
No change shown in sedimentation rate	1	1	0
Data missing	0	1	3
Totals	27	27	27

Table 4 gives a comparison of the sensitivity of the Cutler and Westergren methods in revealing changes observed in the sedimentation rate during menstruation. It will be seen that with the Cutler method 18 out of 27 one-hour readings were increased during menstruation, also 13 two-hour and 11 twenty-four-hour readings. With the Westergren method 21 out of 27 one-hour readings were increased during menstruation, as were 20 two-hour and 16 twenty-four-hour readings. There was fairly good uniformity in the results of the two-hour and twenty-four-hour results. There was disagreement between the two-hour readings in eight cases out of 25, but in the twenty-four-hour readings in only two cases out of 24 for which data were available.

Discussion

It will be seen from the data that has been presented that the sedimentation rate was frequently elevated during the menstrual period, but usually became lower again within a week or so following it. The increases actually found in the SR at this time are so variable, however, that it is necessary to consider just what significance, if any, should be attached to them.

The Cutler method showed an average difference of 0.7 mm. between the SR determined during and after menstruation, a difference so small that it is really more significant to consider individual differences. It will be seen from table 3 that the greatest variation between two comparative tests was 6 mm. for the one-hour reading, a difference great enough to be taken into account in the interpretation of the sedimentation test. A closer analysis of the data in table 1 shows that in about a quarter of the tests, seven to be exact, the increases in the SR during menstruation amounted to 3 to 6 mm. at the end of one hour, while one test showed a decrease of 3 mm. In most of these cases it is doubtful if much significance could be attached to any of these differences, yet if menstruation were taken into account it would provide a reason for these increased rates if no other could be found to account for them. These variations compare fairly closely with those given by Greisheimer (3) who did not consider them significant in her studies on normal women.

In the case of the Westergren results, 13 tests, or about one-half of them, showed increases of 7 to 37 mm. in the one-hour readings while two showed decreases of 13.5 mm. and 17 mm., respectively. Some of the increases were significant and might have indicated greater tuberculous activity unless menstruation had been taken into account. The decrease of 37 mm. noted after menstruation in the case of Miss M. B. was so large that it was wondered if a technical error had been made in one of the tests but that did not seem likely.

One of the two patients showing a moderately decreased SR during the menstrual period had had a pneumonolysis a few days before the tests and almost certainly had some pleural fluid as a result, the formation of which frequently causes a marked rise in the SR. No definite reason could be assigned for the decreased SR shown in the second patient, though earlier tests were higher than either of the two in this series.

It was shown in a previous paper (2) that the average one-hour Westergren reading was 2.48 times the average one-hour Cutler reading. The corresponding Westergren/Cutler ratio found in this smaller series was 9.43, which would seem to indicate that the Westergren technique is more sensitive than the Cutler in reflecting the changes occurring in the sedimentation rate during the menstrual cycle.

Plass and Rourke (9) and Zechwer and Goodell (18) and others have observed that the sedimentation rate is a very constant quantity and usually varies very little from week to week. Many observers have noted the wider normal range in women than in men and for this reason have set their normal standards for women sufficiently high so as to include any variations due to the physiological function of menstruation.

Most of the studies on the subject of the effect of menstruation on the sedimentation rate have been made on normal women while those in this series all had tuberculosis. Whether or not this disease was a factor in causing increases in the SR in the patients in this series is not known.

Increases in the SR during menstruation were found in all stages of pulmonary tuberculosis as will be seen by these examples:

Patient	Pulmonary Tuberculosis	CUTLER TESTS		WESTERGREN TEST S	
		During Mens. mm.	After Mens. mm.	During Mens. mm.	After Mens. mm.
E. W.	Minimal	17	11	36	30
E. C.	Moderately advanced.	17.5	12	37	26
A. I. E.	Far advanced	9	5	19	7.5
M. J. B.	Far advanced	30	26.5	98	87
F. K.	Pleurisy with effusion	15	9.5	33.5	22.5

Miss E. W., mentioned above, had a respiratory infection at the time of the first test but was recovering when the second one was done. This infection may have been an additional factor in causing the moderately wide spread between the results of the two tests within the short period of seven days.

The two-hour and the twenty-four-hour readings showed somewhat similar variations as did the one-hour but the range of variation in either case was slightly less. Table 4 shows that the number of tests with increased sedimentation rates became less as later readings were taken, while the number showing a decreased SR remained either about the same or was slightly increased.

Mrs. D. C. was the only patient on whom sedimentation tests were obtained during two different menstrual periods. Although her SR had been very rapid for some time previously both methods showed slightly lower rates at all reading times in the tests done after the end of the first period. The tests taken during and after the next menstrual period, however, showed exactly the opposite result, i. e., the SR was slightly increased at all reading times as shown by both methods. The only reason that can be given to account for these contrary findings is the fact that several days before the second series of tests was done the patient had had a treatment for a bronchial condition by bronchoscopy which was followed by a general reaction with increase of symptoms for several days.

Summary

1. The sedimentation rate is frequenty elevated during menstruation but the increase is not large, nor is it constant.

2. Increases in the sedimentation rate, as shown by the one-hour readings, were registered by either the Cutler or Westergren methods or both in 23 out of 27 patients, or 85.2 per cent.

3. The average one-hour Cutler reading during menstruation was 15.6 mm., while after the period it was 14.9 mm.; the corresponding Westergren values were 42.7 mm. and 36.1 mm., respectively.

4. The range of variation between the sedimentation rates determined during and after menstruation was 0.5 to 6 mm. for the Cutler method, and 0 to 37 mm. for the Westergren.

5. In only about one-quarter of the Cutler tests and about one-half of the Westergren were the increases in the sedimentation rate during menstruation considered great enough to have significance in the interpretation of the test.

6. The Westergren method appeared more sensitive than the Cutler in revealing changes in the sedimentation rate during menstruation.

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BLOOD BANKS*

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Since this contribution is primarily for the Medical Technologist, it is my intention to stress technic and possible complications, rather than to go into too much detail with reference to the actual operation of the "front office" of the blood bank. However, it is necessary for those who have had no experience, for some thought to be given to these matters. The technologist is, in my opinion, the hub of the wheel, so to speak, and will, in many cases, be asked to start at the beginning and set up the blood bank.

As far back as 1835, whole blood was transfused from one human being to another with success in only a few of the cases. Reactions and deaths from this method, which was simply stirring the blood to prevent clotting, soon caused it to be discarded. We know now, of course, that the cause of these reactions was due to the incompatibility of blood because of the grouping. It was in 1900 that the typing of blood was definitely established and that patients could receive blood from donors of their same type. By 1907, the four types came into the picture. During this seven years, whole blood was given directly from the donor to the patient through needles and short rubber tubing. This was the direct method of transfusion.

In 1914, it was discovered that citrate would keep the blood from clotting and that blood could be drawn into a flask and permitted to stand or be taken even to another room to the patient. This was and is known as the indirect method. Since that time, there have been developed by our commercial laboratories many types of vacuum bottles and equipment which have greatly simplified this important operation.

In about 1932, it was found that citrated blood could be kept in the icebox for as long as ten days without much hemolysis, that the blood was safe to use and the efficiency was almost com-

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plete. Thus the idea of a blood bank was established. It is believed that the first blood bank was that of the Cook County Hospital in Chicago. This bank was followed by hospitals throughout the country until now we have blood banks even in some of the smaller communities throughout the entire nation.

The whole blood bank has its disadvantages in the fact that the blood may be kept only for a period of ten days and special refrigeration is necessary. Since citrated blood will not clot, and the cells will settle to the bottom of the flask, leaving the clear plasma on top, this makes an ideal way of obtaining this precious fluid. When plasma is obtained in this way, it is known as the sedimentation method. Of course, the blood may be centrifuged and the plasma obtained in this manner. Blood plasma was suggested during World War No. I, but it was not until 1938 that it was used in our country.

It has been shown that the pooling of plasma will dilute the agglutinins, which, of course, makes cross matching unnecessary. It has been suggested that plasma when diluted with equal parts of saline is safe for administration but, since we have no way of controlling the titer, and even if we diluted one plasma, we might still have a high titer. Since SAFETY at all costs is our primary objective, it is the general opinion that pooling *should* and *must* be done in justice to patients who entrust their lives to us.

Now in order to set up a blood bank, there should first be a complete plan made for the keeping of records. First, a file must be established for the records of donors, that is the history which must always be taken and kept. The forms to be filled out should be in simple terms so that the lay assistants will be able to take the history, thereby releasing the doctor, nurse, or technologist, for other more important work. When this history is taken, it should be arranged in a file and kept where it can be produced on a moment's notice. The second set of records must be the records on the blood which is taken and issued from the bank. There are many systems for this, and, since in most instances, they are different, it becomes a more or less individual problem. The blood is banked, records made of it, and then when it is issued, records should be made as to whether it was issued as whole blood, or when it has aged and made into plasma, of course, it should be marked

as such. If the cells are retained and used, then records should be made of this. In other words, the blood should be followed through from the donor to the final disposition. Much has been written about advertising the bank and requests for donors. Generally, the pay-off is two donors for one unit of plasma.

In cases of small hospitals or clinics, a donor registry may be kept where the establishment of a bank is not practical.

Preparation of Plasma

All equipment must be chemically clean and sterile. First, we must consider the preparation of this equipment and this is the unseen work which takes the technologist's time but, if it is not properly carried out, then the whole project will be a complete failure.

The rubber tubing may be cleansed and sterilized in many ways so a technologist must decide just which method he chooses and it is good to establish a routine and stick to it.

One method is as follows: *Caution*—All distilled water must be fresh, since pyrogens will develop within a few hours.

New Tubing

1. Boil tubing for thirty minutes or sterilize in an autoclave at 15 pounds pressure from twenty to thirty minutes (the less time you can use under pressure the better, since the rubber will stand up only after a limited number of sterilizings), in a solution of 2 per cent sodium hydroxide. Be sure that the tubing is completely filled with the solution. This may be done by placing one end of the tubing in the solution and slowly lowering it down into the solution so that the air is expelled.
2. Rinse the tubing in tap water.
3. Let tubing soak for 15 minutes in a 1% solution of hydrochloric acid. Again be sure that the tubing is completely filled with the solution.
4. Rinse tubing thoroughly in tap water followed by several washings and rinsings in distilled water.
5. Coil tubing without kinking, wrap in towel and sterilize in autoclave 15 pounds, 15 minutes.

Used Tubing

1. Remove tubing from equipment and wash thoroughly with

tap water.

2. Rinse completely and with care with distilled water.

3. Coil without kinking and sterilize at 15 pounds for 15 minutes.

Tubing That Has Been In Contact With Blood

1. Immediately after use, rinse with cold tap water.

2. To remove all traces of blood, place tubing in solution of 3% sodium bicarbonate and boil 15 minutes. Care should be exercised as above to insure the tubing being filled with solution.

3. Rinse tubing in tap water then rinse thoroughly with fresh distilled water.

4. Coil without kinking and sterilize 15 pounds for 15 minutes.

Metal Equipment and Glassware

Clot filters, needles, aspirating needles, etc.: This equipment must be cleansed thoroughly with tap water, rinsed thoroughly with distilled water and sterilized along with the tubing in the autoclave.

Collecting the Blood

All donors who come to a bank are nervous. There should be a hostess to meet them and make them feel at ease from the start. Care should be taken in the selection of the hostess since she is the first person the donor contacts and it is very important from the standpoint of repeat visits and advertising that the donor is pleasantly impressed from the start. Donor table sshould be comfortable with arm rests. Equipment should be kept out of sight as much as possible and the tables should be so arranged that donors cannot see each other. There may be as many tables as your space will allow. This may be done by the arrangement of the tables or by screens. The blood may be collected by the doctor or nurse. In many cases, the technologist does the bleeding. There is some discussion as to the collecting of blood under vacuum. Some operators release the vacuum and bleed by gravity. It is an individual problem, both methods having advantages and disadvantages.

Bleeding

There are several bottles prepared by commercial houses for bleeding. These bottles are prepared with vacuum and citrate. The

500 cc size with 50 cc of 4% citrate is the one generally used. There are special bottles made for the sedimentation methods and for the different type centrifuges. There are also many types of valves made for the withdrawal of blood. They are very satisfactory but there are many operators throughout the country who use simply a piece of rubber tubing with a needle on either end and a shut-off clamp on the tubing. These bleeding sets are prepared as has been described under tubing.

Obtaining the Blood

Saturate the inside of the bottle with the citrate by rotation. This is very important and insures complete mixing of the blood with the citrate. Check the valve or shut-off clamp. Be sure it is closed. If using a valve, it is sometimes necessary to place a drop of glycerine on the needle adapter before sterilization in order to guard against leaks. Insert the needle in the stopper (generally marked "diaphragm"). Invert the bottle to check for leaks. If the valve is leaking, you will get a series of bubbles from the needle point through the citrate. Scrub the donor's arm with iodine followed by alcohol. Do veni-puncture. The process of withdrawal will become strictly individual with practice. We have found that one good method is to invert the bottle and let the blood flow through the citrate. If this method is used, the valve is regulated so that the blood makes a little geyser about one-fourth to one-half inch above the surface of the citrate. Of course, as more blood is drawn, the rate of flow must be increased. No agitation is necessary with this method. It has been found that a swirling motion is bad. Very little shaking is necessary but there should be a slight rocking motion every once in a while. The vacuum bottles allow a very speedy withdrawal but for the greatest comfort of the donor and for blood most satisfactory for the preparation of the plasma, we have found, and it is the concensus of opinion, that at least seven minutes should be taken to draw 500 cc of blood. Blood should be collected from the donor for typing and serology before the donor is subjected to bleeding. It is necessary, however, that a few cc of blood be collected in a tube which is attached to the donor bottle for cross matching if the blood is to be used for whole blood transfusion. When the blood is collected, it should be placed immediately in a refrigerator at around 4-5 degrees

centigrade. As for whole blood, it is simply taken, kept refrigerated, and administered. It goes without saying that all technics should be under sterile conditions. There has been much discussion and many varied opinions as to the culturing of whole blood. It is advocated in some banks that one bottle from the day's work may be cultured as a check on technics and this would probably be a good practice. Dr. Hill of Baylor University Hospital, Dallas, Texas, cultures every bottle of whole blood.

Method of Obtaining Plasma

As stated before, the plasma may be obtained by centrifuging or by sedimentation method. If the blood is to be centrifuged, the flasks should be carefully balanced and centrifuged at a top speed of around 2,500 rpm. Heating of the blood has not proved too troublesome, since, if the room temperature is kept at 25 degrees centigrade, it has been proved that the rise in the temperature of refrigerated blood is only from 11 to 16 degrees, which, of course, is not harmful. If the blood is at room temperature, the rise in temperature is only approximately one degree. If there is a volume of work being done, which tends to heat the centrifuges, then it is very practical and, almost necessary, to have some method of refrigerating the centrifuges. This is an engineering problem and will not be discussed here. Centrifuging, of course, is faster, and the yield is greater than by the sedimentation method, but the equipment is rather expensive. Sedimentation gives its maximum yield at the end of about seven days. It is ideal for the salvaging of whole blood which is becoming too aged to be used for whole blood transfusion. The yield by this method is within approximately 10% of the centrifuge method.

Pooling

All equipment must be sterilized and the same precautions taken to maintain sterility throughout the procedure as is used in the bleeding of the donor. The best technic is for the person doing the work to wear a gown and mask and the pooling should be done in a private room with no interference. It is always a good policy to pool the bloods according to their types, rather than to merely take random samples. Davenport uses balanced volumes of the various types as follows: 40 to 45 per cent, type A; 10 to 15 per cent, type B; 40 to 45 per cent, type O. Type AB is sometimes

absent because of its rarity or its being present occasionally in a concentration of around 2.5 per cent. The Southern Baptist Hospital of New Orleans had processed 5,500 units of plasma as of January 1, 1944, and the percentage of general reaction rate was less than one-half of one per cent. This is a fine record and it proves that this method of pooling is no doubt conducive to a very small reaction rate. The minimum of blood to be pooled should be eight and it is better, if possible, to pool even more, however, in the small bank, eight is sufficient and much easier to handle with the equipment available. There are many types of valves which may be used for aspirating the plasma from the cells. These valves are all very good but the simplest method is to use a hose hub needle on one end of the tubing with an aspirating needle at the other end. It has been our experience that the aspirating needle with the end closed and a hole on one side is the best one to use, since there is less chance of disturbing the cells and aspiration can be made closer to the cell line. There should be a shut-off clamp of some type on the rubber tubing. It has been found that the aspirating needle should be coated with glycerin and then covered with a piece of penrose tubing before sterilization. The penrose tubing makes it easier to handle the long needle and insures sterility. Some use a very short piece of glass tubing over the sharp end of the needle but this is not necessary. After the blood has been centrifuged, or has been allowed to settle for the maximum number of days, it is taken to the pooling room and the following procedure followed:

Caution: Sterile technic must be used.

Sterilize the stopper of the bottle with iodine. Insert an air filter needle. This may be an ordinary hypodermic needle or it may be desirable to use a needle with a short piece of rubber tubing which has been prepared with a glass tube filled with cotton to be used as a filter at the other end.

Do not place this needle in the airway opening.

Insert the short needle of the aspirating set into the pooling flask. Sterilize stopper. These flasks are generally 2,000 cc capacity with vacuum. Care must be used to insure no leakage around this short needle. Insert the aspirating needle into the bottle containing the plasma. Here the point of the aspirating needle is placed

about halfway through the plasma and the valve or shut-off clamp opened. Watch carefully and when the surface of the plasma has just about reached the needle point, push the needle further down and so on until the cell line is reached. Now, remove the needle carefully and place it in the next bottle. Continue this until the eight bottles have been aspirated. *Mix the pool well.* With a sterile 10 cc cyringe, remove 10 cc of plasma. Place 5 cc into about 35 cc of Brewer's thioglycollate medium and 5 cc in nutrient broth. In most of the banks that I have visited, it is routine to use several tubes of media for each culture. We have found that there are some artefacts in the media occasionally having the appearance of bacteria. With practice, this will not be bothersome, but to the novice, it sometimes causes a great amount of excitement. Another thing of interest that we found in one pool was a rather pronounced veil formation in the culture. After making many smears, and transplants, we were unable to demonstrate bacteria. The veil had the appearance of a slight growth in the culture but, on diluting the plasma on this particular pool, we were no longer bothered with this phenomena.

At this point, preservatives may be added to the pool. Merthiolate is the preservative generally used. Phenylmercuric borate is used in many places. The preservative is made up as follows: Make a one per cent solution and autoclave for 30 minutes at 20 pounds. Store in a dark bottle. This is your stock solution. If merthiolate is used, add one cc of this stock for each 100 cc of plasma. This gives a final dilution of one to 10,000. If phenylmercuric borate is used, then .6 cc of this stock for each 100 cc of plasma will give final dilution of one to 15,000.

Allow the pool to stand in a refrigerator while waiting for the culture. If, at the end of seven days, there is no growth in the culture, the plasma is ready to dispense into the bottles for distribution. Technic for this is the same as the technic used in pooling, except that the aspiration needle is placed in the pooling bottle and plasma is aspirated into the small bottles. Two hundred and fifty cc is considered a unit and allowances should be made for the citrate present in the plasma. Label the last bottle taken from the pool "pilot." After the pilot has stood over night, at room temperature, take a culture and place 5 cc of the pilot in

each of two tubes of media, incubating one at room temperature and one at 37½ degrees centigrade. If pilot is negative at the end of two weeks, plasma is ready for use.

The hemoglobin content of plasma has been the source of much argument. In my rounds of the blood banks throughout the country, I have especially tried to find a simple method that would be practical for the small bank, however, there is no really simple method for a bank which has no chemical control laboratory to do the work. Much has been said about danger of hemoglobin and it is good practice to keep the hemoglobin down as much as possible. Some authorities claim that the hemoglobin content of plasma should be less than twenty-five milligrams per 100 cc. After the plasma is made, the question of storage comes into the picture. There are several methods for storing, namely:

(1) Liquid plasma kept at room temperature. This has the advantage of convenience and there is less fibrin veil formation. Most of the elements are in their native states. The disadvantage is that it loses its complement within a very short while. If the technic has not been proper, and a contaminant has entered, it will multiply when stored at this temperature.

(2) Refrigeration advantages are that complement remains a little longer than when stored at room temperature. Disadvantages are that fibrinogen is quickly converted to fibrin which gives undesirable appearances. The heavy fibrin veil presents tremendous filter difficulty upon infusion. The buffer action and the prothrombin remain in the filter which is very bad if the recipient is in need of these elements.

(3) Frozen, the advantages are that elements are in their native states. There is absolutely no veil formation if the frozen product is thawed properly. Another advantage is perfect keeping qualities. Disadvantages—there is considerable expense and trouble in freezing. It takes a considerable amount of time to melt properly before using and is limited to use in one institution unless transported in frozen condition.

(4) Dried or desiccated advantages: All elements are in their native states, complement remains indefinitely if plasma is processed immediately after it is prepared and having perfect keeping qualities, it may be stored under almost any conditions. It is prac-

tical for some very isolated rural communities where it might be stored indefinitely. Disadvantages: Bottle is open so much during processing that it is much more subject to contamination than plasma prepared with a closed method. It is very difficult to make requiring much equipment and more skilled technicians. It is at least one-third more expensive than other products.

Here let me discuss dry plasma briefly. There are several methods for drying plasma. The three most commonly used are the steam jet method, the cold surface method, and the silicagel. These methods are all good, but dried plasma should be prepared only in banks or hospitals which are big enough to supply chemical control laboratories where the moisture, hemoglobin, protein, and titer agglutination determination can be made. Of course, the protein should be done on all plasma. It is well to do titer agglutination also. These examinations are the same as are used in any clinical laboratory, but it is not practical to do hemoglobins in the small bank until simpler methods can be found. Plasma should be tested for toxins. This is generally done by the innoculation of animals.

To complete this paper, since the title is "Blood Banks," I should mention red cells. In some laboratories and banks throughout the country, the physicians are calling for red cells only. This simply means that the red cells are saved from the bottles after the plasma has been aspirated. The cells are resuspended in saline and administered to the patient by infusion. Only type "O" cells are being used by the Red Cross and naturally there are rigid rules as pertaining to sterility and age of the blood. No blood is used after five days.

In closing, let me say that I feel that the medical technologists should be especially proud of the part they are taking in this important work, since, in my opinion, they are the best qualified persons to undertake the complete operation. This work requires a knowledge of hematology, serology, bacteriology, bio-chemistry, patience, and psychology. Put all these together, look for someone who can qualify, and you have a medical technologist.

I would like to express deep gratitude to the following for their interest and suggestions:

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ABSTRACTS

THE EFFECT OF SODIUM HYDROXIDE ON BIOLOGIC FALSELY POSITIVE AND ANTICOMPLEMENTARY REACTIONS IN SYPHILIS: H. Brown, J. A. Kolmer and E. R. Lynch, Am. Jr. Syph., Gon. & Ven. Dis., Vol. 29, No. 2, Mar., '45, p. 200.

The addition of 0.1 ml. of N/3 or N/4 NaOH to 1 ml. serum before inactivation at 55° C. has been effective in eliminating falsely positive and inticomplementary Kolmer, Kahn and Mazzini tests in blood from rabbits and blood from humans with malaria, infectious mononucleosis and virus pneumonia. The N/3 was more effective than N/4.

In serum from lepers results were less favorable. The N/4 was better than N/3 NaOH.

This treatment also destroys some of the syphilis reagin but false negatives were only obtained where the serum contained small amounts of reagin.

PRIMARY SPLENIC NEUTROPENIA WITH REPORT OF A CASE: M. Salzer, J. L. Ransohoff and H. Blatt, Ann. Int. Med., Vol. 22, No. 2, Feb., '45, p. 271.

Patient was first seen with the complaint of chills and fever. She had had pneumonia 11 months before. At that time her blood showed RBC 4,970,000, WBC 2,750 with 24% polys. She had never completely recovered from this illness. When seen now her RBC was 4,700,000, WBC 2,000 with 50% polys. The diagnosis was again pneumonia. Her WBC dropped to 800 with 36% polys. Sternal puncture smears were normal. The spleen was enlarged. Sulfadiazine was given with favorable results. She subsequently showed improvement but 2 weeks later developed pleurisy. At this time the spleen was again palpable. She again recovered. Approximately one month after her original admission a splenectomy was performed. Two days later the WBC was 14,650 with 87% polys. Four months later she had a WBC of 12,450 with 76% polys and appeared perfectly well.

SENSITIVITY OF BACTERIA FROM INFECTED WOUNDS TO PENICILLIN: E. Gallardo, War Med., Vol. 7, No. 2, Feb., '45, p. 100.

Among the 108 strains of staphylococci studied, 22.2% were naturally resistant to penicillin or became so during the course of treatment. Eleven pathogens and 4 non-pathogens showed natural resistance. Eight pathogens and 1 non-pathogen developed penicillin fastness. All these cases had previously been treated with sulfa drugs.

As penicillin-fastness developed morphological changes as well as a tendency to become Gram negative were seen. Culturing in tryptone-phosphate broth returned the normal staining reaction in every instance.

A routine study of bacterial sensitivity in all infections is advised. This should be done before the administration of penicillin and repeatedly during the course of treatment to determine future treatment.

THE ABSORPTION OF SULFONAMIDES FROM THE BURN SURFACE: E. I. Evans, M. J. Hoover & G. W. James, Surg., Gyn. & Obstet., Vol. 80, No. 3, March '45, Pp. 297.

Sulfa drugs in a water dispersible base were applied in four burn cases. Blood levels of 20.4 mgs.%, 28 mgs.%, 10 mgs.%, and 21 mgs.% were recorded. Using the same procedures on four other cases but applying the sulfa drugs in an oil base, 2.8 mgs.% was the highest blood level obtained.

Observations indicate that sulfa drugs administered in an oil base are much safer than the same drug administered in a water dispersible base. A low incidence of infection is reported for them.

A COMPARISON OF GASTRIC ACIDITY AMONG MEN AND WOMEN SUFFERING FROM DUODENAL ULCER AS DETERMINED BY FRACTIONAL ANALYSIS OF GASTRIC CONTENTS AFTER INJECTION OF HISTAMINE: G. E. Brown & A. B. Rivers, Am. Jr. Diges. Dis., Vol. 12, No. 2, Feb. '45, Pp. 33.

The average maximum free HCl obtained for men was 90 ml. N/10 NaOH and for women 67 ml. Total acidity values were 103 ml. for men and 80 ml. for women. The women who had values exceeding 100 ml. were extremely nervous and gave poor response to treatment. This was in keeping with the poorer responses generally obtained in the male.

MALARIA FROM BLOOD BANK TRANSFUSIONS: R. D. McClure & C. R. Lam., Surg., Gyn., & Obstet., Vol. 80, No. 3, March '45, Pp. 261.

Two cases of malaria from bank blood refrigerated as long as five days are reported. Both donors were men who had spent their childhood in the same small town in Sicily. One had a history of chills and fever at age 10. The other had none. Both had lived in Michigan entirely without malarial symptoms for at least 25 years.

Since the addition of quinine to the blood is without effect and no means of preventing transfer is available, it is suggested that whenever possible the blood of donors who have lived in malarial areas be converted to plasma if it must be used.

CHOLELITHIASIS IN SICKLE CELL ANEMIA: H. S. Weens, Ann. Int. Med., Vol. 22, No. 2, Feb., '45, p. 182.

Case reports of 4 negroes are given in which cholecystograms disclosed gall-stones. The literature reviewed indicates that the biliary calculi occur less frequently in the negro than in the white race, they do occur more frequently in those suffering from sickle cell anemia. This increased incidence is particularly evident in the age groups below 40 years.

There is evidence to show that an excess of bilirubin in the bile favors the precipitation of pigment in the biliary tract. Since there is increased destruction of RBC with consequent increased production of bilirubin in sickle cell anemia, this may favor formation of gall-stones.

The author stresses the need for careful search for sickle cell anemia when cholelithiasis occurs in a negro, particularly in a young patient.

INTERPRETATION OF THE COLIFORM TEST: Bull. U. S. Army Med. Dept., Vol. IV, No. 1, July '45, p. 43.

Since it is more important to know whether an excessive number of coliform organisms is present than to know the exact number, the following presumptive test is proposed. One ml. of the milk sample is added to each of 5 tubes of lactose broth. If more than 2 of the 5 show gas, the sample is considered positive. If only 1 or 2 show gas, this is taken to be insignificant contamination.

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The Ohio Society of Medical Technologists will have its first meeting with the new officers in September at Children's Hospital.

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The Texas Society of Medical Technologists will not hold an annual convention this year in accordance with the O.D.T. travel regulations.

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1945-1946

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